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<p>(21) International Application Number: PCT/CA97/00424 (22) International Filing Date: 16 June 1997 (16.06.97) (30) Priority Data: 60/020,553 26 June 1996 (26.06.96) US (71) Applicant (for all designated States except US): MCGILL UNIVERSITY [CA/CA]; 845 Sherbrooke Street West, Montréal, Québec H3A 1B1 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): BROWN, Gregory, G. [US/CA]; 4990 West Broadway, Montréal, Québec H4V 1Z9 (CA). LANDRY, Benoit [CA/CA]; 133 Allée des Cigales, L'Acadie, Québec J3J 1H0 (CA). JEAN, Martine [CA/CA]; 877 Delage #3, Sainte-Foy, Québec G1V 3X3 (CA). (74) Agent: CÔTE, France; Swabey Ogilvy Renault, Suite 1600, 1981 McGill College Avenue, Montréal, Québec H3A 2Y3 (CA).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE AND NUCLEAR RESTORATION OF CYTOPLASMIC MALE STERILITY</p>		
<p>(57) Abstract</p> <p>The present invention relates to a marker for nuclear restoration of cytoplasmic male sterility, and more particularly to the use of glyceraldehyde-3-phosphate dehydrogenase complementary DNA as such a marker. There is provided a gene for nuclear restoration of cytoplasmic male sterility, and more particularly to the use of a form of the gene encoding glyceraldehyde-3-phosphate dehydrogenase for this purpose. Finally, there is provided a method for the production of restorer lines directly through genetic transformation of plants with such a gene.</p>		

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**GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE AND NUCLEAR
RESTORATION OF CYTOPLASMIC MALE STERILITY**

BACKGROUND OF THE INVENTION

5 (a) Field of the Invention

The invention relates to a marker for nuclear restoration of cytoplasmic male sterility, and more particularly to the use of glyceraldehyde-3-phosphate dehydrogenase complementary DNA as such a marker. The
10 invention also relates to a gene for nuclear restoration of cytoplasmic male sterility, and more particularly to the use of a form of the gene encoding glyceraldehyde-3-phosphate dehydrogenase for this purpose. Finally, the invention relates to the production of
15 restorer lines directly through genetic transformation of plants with such a gene.

(b) Description of Prior Art

Hybrids of different crop varieties may show yields that are considerably greater than those of the
20 parental lines. This phenomenon is known as hybrid vigor. To implement the use of hybrid vigor it is necessary to have a method available for preventing self-pollination of one or both of the parent lines in the hybrid cross. Mechanical, chemical and genetic
25 methods are available for accomplishing this. One established genetic method involves the trait of cytoplasmic male sterility (CMS). The genetic determinants for CMS, the maternally transmitted inability to produce viable pollen, reside on the mitochondrial genome.
30 Because CMS plants are male sterile, all of the seed that forms on them will necessarily be hybrid. Due to the maternal transmission of CMS, however, such F1 hybrids will also normally be male-sterile and hence be unable to self-fertilize and produce seed. To address
35 this problem, specific nuclear genes that suppress the male sterile phenotype, termed restorers of fertility

(Rf), can be incorporated into the pollinating parent of the hybrid cross. Genotypes on which the male sterile cytoplasm confers sterility are termed maintainers whereas those carrying Rf genes are termed restorers; 5 the genes for the maintenance and restoration of CMS can be considered as different alleles (rf and Rf, respectively) at the same locus.

Shortcomings of present solutions

To produce a diverse set of hybrids using CMS, 10 adequate numbers of restorer lines, that contain Rf genes, as well as "maintainer" lines, that are sterilized by the CMS cytoplasm, must be available. The use of such lines in hybrid crop production is outlined in Fig. 1. The development of these lines through conven- 15 tional genetics is a slow process that minimally requires several years of effort and currently poses a major bottleneck in the generation of CMS-based hybrids in a number of crops, including canola, Canada's major cash crop. For example, to create a new restorer line 20 it is necessary to first generate a hybrid between an existing restorer strain, which donates the Rf gene, and a recipient strain; a series of backcrosses to the recipient strain are then performed to incorporate the Rf gene without altering the strain's other desirable 25 characteristics, a process termed introgression. Even after many generations some donor DNA that is linked to the Rf gene on the donor DNA will remain, a phenomenon termed linkage drag; this donor DNA may carry deleterious traits and compromise the quality of the recipient 30 strain (Jean, M. et al., 1993, *Current Topics in Molecular Genetics*, 1:195-201).

This process can be expedited through the general process of indirect selection: progeny plants are first screened for genetic markers linked to the 35 restorer gene rather than the restorer gene itself.

These markers are chosen such that they can be screened for at a very early stage in plant development. This circumvents the costly procedure of raising many progeny plants to maturity and can considerably accelerate the introgression process. Restriction fragment length polymorphisms (RFLPs) represent a type of DNA marker that is ideally suited for this purpose. RFLPs are differences (between two genotypes) in restriction fragment patterns detected by specific DNA probes. Probes that detect fragment pattern differences between restorer and maintainer lines and that co-segregate with the *Rf* gene can be used to indirectly select for the restorer gene in a plant breeding program. We have obtained several probes that are linked to *Rfp1*, a restorer of the Polima or *pol* CMS, one of the two forms of CMS in canola (*B. napus*) that is currently being used in hybrid seed production. None of these markers is completely linked to the gene. This introduces an element of uncertainty into their use for indirect selection-the presence of any one marker in a plant does not guarantee the presence of the restorer gene in that plant. It therefore is necessary to employ a number of the markers for indirect selection of plant containing the restorer gene.

It would be highly desirable to be provided with a marker that is perfectly associated with nuclear restoration of cytoplasmic male sterility.

This process can be further expedited through direct introduction of a cloned restorer gene. We believe that the probe we have identified, which show perfect linkage to *Rfp1* is detecting the restorer gene itself.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide a marker for nuclear restoration associated with cytoplasmic male sterility.

5 Another aim of the present invention is to provide the use of glyceraldehyde-3-phosphate dehydrogenase complementary DNA as such a restorer marker.

Another aim of the present invention is to be able to use this gene to produce restorer lines
10 directly through genetic transformation.

In accordance with the present invention there is provided a probe specific for nuclear restoration of cytoplasmic male sterility of plants, which comprises a glyceraldehyde-3-phosphate dehydrogenase cDNA or
15 genomic DNA sequence, a hybridizing fragment thereof or any DNA sequence derived therefrom for use as primers for amplification of glyceraldehyde-3-phosphate dehydrogenase, wherein said DNA sequence or hybridizing fragment thereof hybridizes to specific DNA fragments
20 characteristic of plants possessing a nuclear restorer gene under stringent conditions.

In accordance with the present invention there is also provided a gene for nuclear restoration of cytoplasmic male sterility in plants which comprises a
25 DNA sequence encoding glyceraldehyde-3-phosphate dehydrogenase and surrounding sequences.

The surrounding sequences may be located 3' and/or 5' relative to the glyceraldehyde-3-phosphate dehydrogenase sequence and may be of about 50kb.

30 In accordance with the present invention there is also provided a method of production of restorer lines, which comprises genetically transforming plants with the nuclear restoration of cytoplasmic male sterility gene of the present invention.

In accordance with the present invention, any plant species may be used provided that the restorer gene in the plant species corresponds to a specific form of GAPC. Such species include, without limitation, *Brassica napus*, other *Brassica* species, maize (*Zea mays*), rice (*Oryza sativum*), sunflower (*Helianthus annuum*) and sorghum (*Sorghum bicolor*).

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic representation of the use of cytoplasmic male sterility (CMS) in hybrid seed production;

Fig. 2 shows the crosses used to identify a marker completely linked to the *Rfpl* restorer of fertility gene;

Figs. 3A to 3E show the comparison of *Brassica napus* cDNA clone cRF1 (SEQ ID NO:1) with cytoplasmic glyceraldehyde-3-phosphate dehydrogenase (GAPC) cDNAs from *Sinapis alba* (SEQ ID NO:2) and *Arabidopsis thaliana* (SEQ ID NO:3); and

Fig. 4 illustrates a gel of the polymorphism detected by cRF1 probe in *Brassica napus* in a genetic population segregating for the *Rfpl* gene.

DETAILED DESCRIPTION OF THE INVENTION

We continued an analysis of two genetic crosses which gave rise to plant populations in which the restorer gene was segregating (outlined in Fig. 2). In each case, the nature of the cross was such that for linked markers, most sterile progeny individuals would show the RFLP characteristic of the male sterile parent of the cross, while most male fertile progeny plants would show the RFLP characteristic of the fertile parent. A new marker, designated cRF1, was found that is perfectly linked to this gene. Specifically, of the 175 individuals tested in the two crosses, all fertile

progeny were found to possess the allele (or form) of the fertile parent while all sterile plants were found to possess the allele of the sterile parent (Table 1). cRF1 therefore represents a particularly powerful tool
 5 for indirect selection of the restorer gene.

Table 1

Co-segregation of an <i>Rfp1</i> -specific RFLP allele detected by the probe cRF1(GAPC) with male fertility restoration in 2 <i>Brassica napus</i> backcross populations				
Cross	Fertile progeny plants		Sterile progeny plants	
	with <i>Rfp1</i> -specific cRF1 allele	without <i>Rfp1</i> -specific cRF1 allele	with <i>Rfp1</i> -specific cRF1 allele	without <i>Rfp1</i> -specific cRF1 allele
Westar x Westar-Rf	30	0	0	34
Karat x Westar-Rf	58	0	0	55
Total	88	0	0	89

Points of difference with previous solutions

10 Because of the perfect linkage between cRF1 and *Rfp1*, the uncertainty in the use of this probe for indirect selection of the restorer gene is virtually eliminated.

15 In addition, no restorer gene for the Polima or *pol* CMS system has been isolated and hence production of restorer lines directly through genetic transformation is not possible. This should result in a significant reduction of the cost of the use of indirect selection in the development of new restorer (Fig. 4)
 20 lines.

The DNA probe that detected this polymorphism is a *B. napus* complementary DNA (cDNA), i.e., a DNA complementary to a messenger RNA molecule (mRNA). The DNA sequence of this cDNA was determined. Analysis of a
 25 nucleotide sequence database indicated that the cDNA's sequence is 99% similar to that of a cytoplasmic form of a glycolytic enzyme from *Arabidopsis thaliana*,

glyceraldehyde-3-phosphate dehydrogenase (Figs. 3A and 3B), which is encoded by the GAPC gene (Shih, M.-C. et al., 1991, *Gene*, 104:133-138). The perfect linkage between the restorer gene and the GAPC polymorphism leads us to believe that the restorer gene is likely to be specific form of GAPC.

We have conducted a similar type of analysis on a BCl population in which the restorer gene for a different *B. napus* CMS, the *nap*, system was segregating and found that the *nap* restorer was simply a different allele of the same genetic locus. Thus different forms of GAPC correspond to two different nuclear fertility restorer genes in *B. napus*. This result further suggests that other restorer genes may correspond to GAPC isoforms and that the relationship between GAPC and restorer genes may extend to other CMS systems in other plant species. No relationship between GAPC and restorer genes for any plant species has been suggested previously.

With this gene it may therefore be possible to construct restorer lines in a single step by using genetic transformation to introduce the restorer-specific GAPC gene into maintainer genotypes (genotypes that do not naturally contain the restorer). This would be extremely cost effective as it would eliminate many steps in the plant breeding process necessary for the development of such lines. If the association between GAPC and restorer genes is extended to other crop species, this would represent a general method for the isolation of restorer genes and the development of restorer lines in many crops.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I

Use of a GAPC probe as an indirect selection marker in the production of a new restorer cell line

Three plant genotypes will be considered:

- 5 A a CMS line;
- B a male fertile line that lacks the restorer gene and contains a male fertile cytoplasm; and
- R a male fertile line that contains the restorer gene and a male sterile cytoplasm.

- 10 It will be assumed that hybrids between lines A and B that are produced by manual genetic crosses show considerable hybrid vigour; hybrids between A and R do not. As line B lacks a restorer gene, it is not possible to produce male fertile hybrids of these two lines
- 15 using CMS. If, however, the restorer gene could be transferred from line R to line B without otherwise altering the characteristics of line B, it would be possible to obtain male fertile hybrids between lines A and B using CMS. Traditionally, this would be done
- 20 through a process termed introgression. Line R is crossed as a female with line B to produce a male fertile F1 hybrid of A and B that contains the male sterile cytoplasm (the cytoplasm of a hybrid is derived exclusively from the female parent) but is also male
- 25 fertile because it has received a single copy of the restorer gene from the line R parent. A second cross (termed a backcross) is then performed between the hybrid (as female) and the line B. Large numbers of progeny grown are in the field, and equal numbers of
- 30 steriles and fertiles are expected, fertiles possessing the restorer gene. One or more fertiles are then used as females in a second backcross to line B; fertile plants are recovered and crossed as females to line B for a third time. This process is repeated for many
- 35 generations; with each new generation the progeny are

expected to become more similar to line B (except they will possess the restorer gene). At each generation various characteristics associated with line B will be assessed. Eventually, new restorer line, with all or most of the desirable characteristics of line B will be produced. This line could then be used for the large scale production of hybrids between lines A and B.

The GAPC probe facilitates this process because it allows for the assessment of the presence of the restorer gene in progeny plants at the seedling stage. DNA is extracted from a small amount of leaf material, digested with a restriction endonuclease, such as *HindIII* (used in Fig. 4) and analyzed using the GAPC probe. The presence of the restriction fragment characteristic of the restorer gene indicates that the seedling has the restorer gene. Very large numbers of plants at the seedling stage are screened at much lower cost than the cost of raising the same plants to maturity in the field. In addition, the male fertile phenotype is affected by many different conditions and screening for the presence of the gene by screening for a perfectly linked polymorphism more reliably detects the presence of the gene during this introgression procedure.

25 EXAMPLE II

Production of new restorer cell lines through the introduction of the restorer gene form of GAPC via transformation

The three plant genotypes of Example I will be considered in accordance with this procedure.

In this example, the problem is precisely the same as that of Example I, namely the transfer of the restorer gene from line R into line B without otherwise altering the characteristics of line B. In this case, however, we will assume that the form of the GAPC gene that represents the restorer gene has been isolated and

is available as a cloned DNA segment in a suitable plant *Agrobacterium tumefaciens* transformation vector such as pRD400 (Datla RSS, Hammerlindl JK, Panchuk B, Pelcher LE & Keller W. (1992) Gene 211:383-384).

5 Instead of the lengthy backcrossing program described in Example I, the GAPC gene is transferred to line B through *Agrobacterium*-mediated transformation.

For the sake of this example, we will also assume that lines A, B and R are *Brassica napus* lines, and that the cloned restorer gene is identical to that of line R. Using the procedure described by Moloney et al. (Moloney, M., Walker, J. & Sharma, K. (1989) Plant Cell Rep. 8:238-242) an *Agrobacterium* strain harboring the gene in the pRD400 vector is used to inoculate cotyledons from strain B seedlings. The *Agrobacterium* is eliminated by antibiotic treatment and the resulting plant tissue is placed on media containing the antibiotic kanamycin. pRD400 contains a gene that confers resistance to kanamycin, and hence cells that grow on this antibiotic are likely have acquired the kanamycin gene, along with the restorer gene which is cloned into pRD400. The presence of the restorer gene in these plants is then assessed directly by testing the plants for the presence of restriction fragments characteristic of the restorer using a GAPC probe. It is expected that these plants will be made fertile if they contain the male sterile cytoplasm and that F1 progeny from a cross between line A (as female) and the new transgenic line will also be male fertile.

30 This method has two distinct advantages: it is much faster and cheaper than conventional plant breeding approaches, requiring only a few months as opposed to years to develop this line. In addition, the presence of the restorer gene will be the only difference between the genome of line B and that of the new

35

restorer line. Thus the integrity of the characteristics of line B are less likely to be compromised.

Although the above description relates to a specific plant species, *Brassica napus*, the invention
5 could be applied to other species provided that the restorer gene in the species corresponds to a specific form of GAPC. In such cases the technique for transformation may differ from that described above.

While the invention has been described in connection with specific embodiments thereof, it will be
10 understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and
15 including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended
20 claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

- (i) APPLICANT: MCGILL UNIVERSITY et al.
- (ii) TITLE OF THE INVENTION: GLYCERALDEHYDE-3-PHOSPHATE
DEHYDROGENASE AND NUCLEAR RESTORATION OF CYTOPLASMIC MALE
STERILITY
- (iii) NUMBER OF SEQUENCES: 3
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 - (E) COUNTRY: Canada
 - (F) ZIP: H3A 2Y3
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette
 - (B) COMPUTER: IBM Compatible
 - (C) OPERATING SYSTEM: DOS
 - (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 60/020,553
 - (B) FILING DATE: 26-JUN-1996
- (viii) ATTORNEY/AGENT INFORMATION:
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 - (C) REFERENCE/DOCKET NUMBER: 1770-152"PCT" FC
- (ix) TELECOMMUNICATION INFORMATION:
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 - (B) TELEFAX: 514-288-8389
 - (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1207 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCTCGATCTC	ATCGACACCC	TCTGATATCG	AAATGGCTGA	CAAGAAGATT	AAGATCGGAA	60
TCAACGGTTT	CGGAAGAATC	GGTCGCTTGG	TGGCTAGAGT	TATCCTTCAG	AGGAACGATG	120
TTGAGCTCGT	CGCTGTTAAC	GACCCCTTCA	TCACCACCGA	GTACATGACG	TACATGTTTA	180
AGTATGACAG	TGTTCACGGT	CAGTGGAAAG	ACAACGAGCT	CAAGGTTAAG	GATGAGAAGA	240
CACCTTCTCT	CGGTGAGAAG	CCTGTCACTG	TTTTCGGCAT	CAGGAACCCT	GAGGATATGC	300
CCATGGGGTG	AGGATGGAGC	TGACTTTGGG	GTTGAGTCTA	CTGGTGTCTT	CACCGACAAG	360
GACAAGGCTG	CTGCTCACTT	GAAGGGTGGT	GCGAAGAAAG	TTGTCATCTC	TGCACCAAGC	420
AAAGATGCTC	CCATGTTTCG	TGTTGGTGTC	AATGAGCATG	AGTACAAGTC	TGATCTCAAC	480
ATTGTTTCCA	ACGCTAGTGC	ACCACTAACT	GCCTTGCTCC	ACTTGCCAAG	GTATCANCG	540
ACAGGTTTGG	AATTGTCGAG	GGACTCATGA	CCACCGTCCA	CTCTATCACT	GCAACTCAGA	600
AGACAGTTGA	TGGTCCATCA	ATGAAGGACT	GGAGAGGTGG	AAGAGCCGCT	TCCTTCAACA	660
TCATTCCCAG	CAGCACCGGA	GCTGCCAAGG	CTGTCGGAAA	GGTTCCTCCA	CAGCTCAACG	720
GAAAGCTGAC	CGGTATGTCC	TTCCGTGTTT	CCACCGTTGA	TGTTTCAGTT	GTTGACTCAC	780
GGTTAGACTC	GAGAAAGCTG	CAACCTACGA	TGAAATCAAG	AAGGCTATCA	AGGAGGAATC	840
TGAGGGCAAG	CTAAAGGGAA	TCCTTGGTTA	CACAGAGGAT	GATGTTGTCT	CAACCGACTT	900
CGTTGGTGAC	AACAGGTCGA	GCATTTTGA	CGCAAAGGCT	GGAATCGCGT	TGAGTGACAA	960
CTTTGTGAAG	CTGGTGTCTG	GGTACGACAA	CGAATGGGGT	TACAGTACCC	GTGTGGTCGA	1020
CTTGATCATT	CACATGTCCA	AGGCCTAAGT	CGATGAAGAT	CTCGAGTGAT	GTAATGGTGT	1080
TTTTAAATTG	TTGTTTTTAT	CGAATAAATT	TTCTTGGGTT	TTGAAACCTT	TATGGTTTTG	1140
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TTACTGA						1207

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1091 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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GAAGCACAAT	GAGCTCAAGG	TGAAGGATGA	GAAAACACTT	CTCTTCGGAG	AGAAGCCTGT	240
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CTTTGACGCC	AAGGCTGGAA	TCGATTGAG	TGACAACCTC	GTGAAGCTGG	TGTCGTGGTA	960
TGACAACGAA	TGGGTTTACA	GTACCCGTGT	GGTCGACTTG	ATCATTGATA	TGTCCAAGGC	1020
CTAAACGCT	GAGATCTAC	AATGATGTAA	TGGTGTCTTA	ATTGTGGTT	TTCGAATAAG	1080
ATTTCTTTGG	G					1091

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1295 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

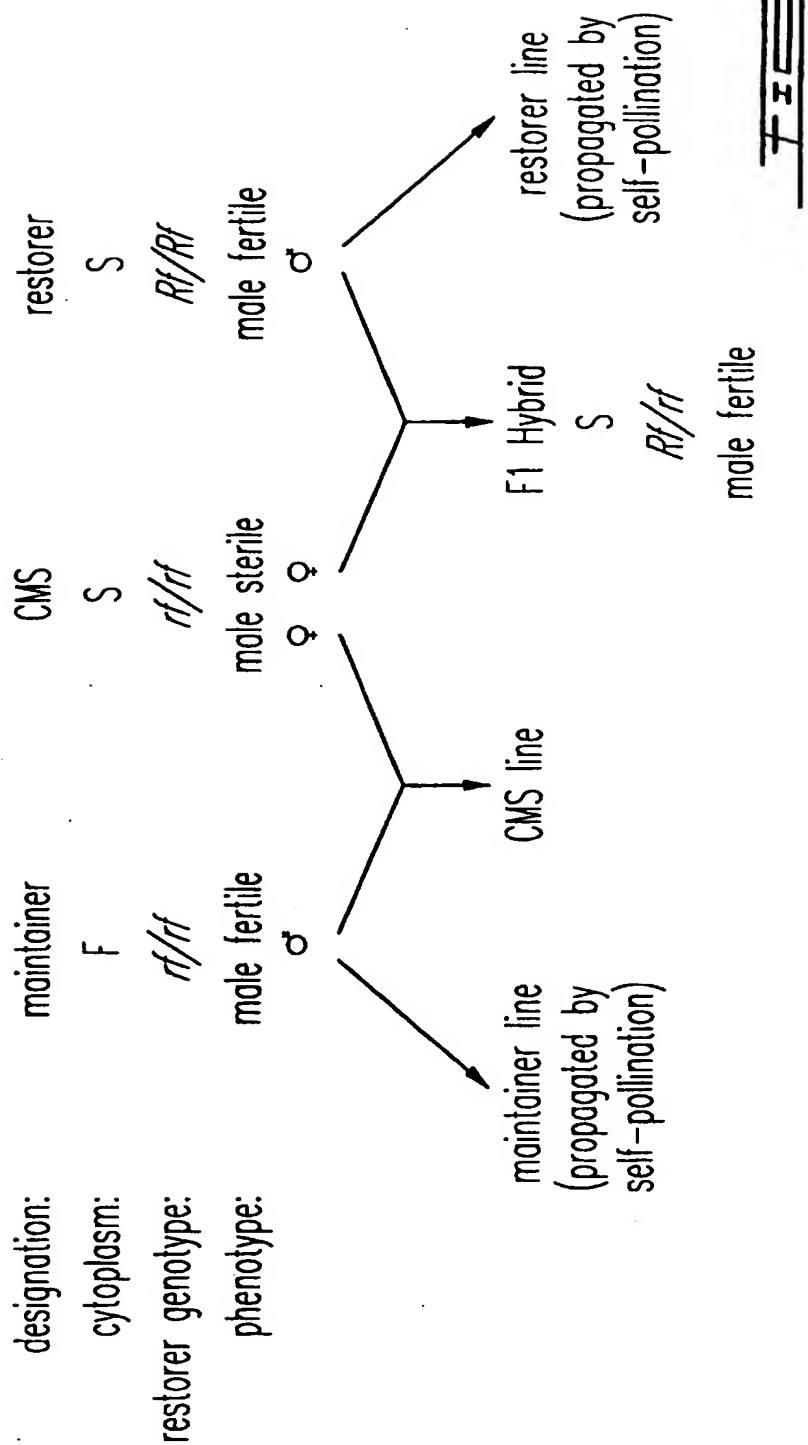
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTCATCTTCA	ACCTCTCTCT	AACTCTCGTT	TTCGATTCTA	CAATGGCTGA	CAAGAAGATT	60
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AGGGACGATG	TTGAGCTCGT	CGCTGTCAAC	GACCCCTTCA	TCACTACTGA	GTACATGACC	180
TACATGTTCA	AGTACGACAG	TGTTACGGT	CAATGGAAAC	ACAATGAACT	CAAGATCAAG	240
GATGAGAAGA	CCCTTCTCTT	CGGTGAGAAG	CCAGTCACTG	TTTTCGGCAT	CAGGAACCTT	300
GAGGATATCC	CATGGGCCGA	GGCTGGAGCT	GACTACGTTG	TTGAGTCTAC	TGGTGTCTTC	360
ACTGACAAAG	ACAAGGCTGC	AGCTCACTTG	AAGGGTGGTG	CCAAGAAGGT	TGTTATCTCT	420
GAACCCAGCA	AAGACGCTCC	AATGTTTGTT	GTTGGTGTCA	ACGAGCACGA	ATACAAGTCC	480
GACCTTGACA	TTGTCTCCAA	CGCTAGCTGC	ACCACTAACT	GCCTTGCTCC	CCTTGCCAAG	540
GTTATCAATG	ACAGATTGGG	AATGTTTGAG	GGTCTTATGA	CTACAGTCCA	CTCAATCACT	600
GCTACTCAGA	AGACTGTTGA	TGGGCCTTCA	ATGAAGGACT	GGAGAGGTGG	AAGAGCTGCT	660
TCATTCAACA	TTATTCCCAG	CAGCACTGGA	GCTGCCAAGG	CTGTCCGAAA	GGTGCTTCCA	720
GCTCTTAACG	GAAAGTTGAC	TGGAATGTCT	TTCCGTGTCC	CAACCGTTGA	TGTCTCAGTT	780
GTTGACCTTA	CTGTCACTAG	CGAGAAAGCT	GCTACCTACG	AAGAAATCAA	AAAGGCTATC	840
AAGGAGGAAT	CCGAAGGCAA	ACTCAAGGGA	ATCCTTGGAT	ACACCGAGGA	TGATGTTGTC	900
TCAACTGACT	TCGTTGCGCA	CAACAGGTCG	AGCATTTTTG	ACGCCAAGGC	TGGAATTGCA	960
TTGAGCGACA	AGTTTGTGAA	ATTGGTGTCA	TGGTACGACA	ACGAATGGGG	TTACAGTTCC	1020
CGTGTGGTCG	ACTTGATCGT	CCACATGTCA	AAGGCCTAAG	CTAAGAAGCA	GATCTCGAAT	1080
GATAGGGAGT	GGAAAGTCAT	CTGTTTCATCC	CCTTTTATGG	TCTGAATTTG	TCGTTTTCGA	1140
ATAAAATTTT	TTTGAACCTG	GAACTTTTTT	TTTTTTTGGT	TTTCTTAATT	CTCATTTCATG	1200
TGAGGTGATG	GGAGTTTGTA	GACCGATGTT	TTACTGGAAG	CCCTTTGTTT	TTGGCTTTTG	1260
ATATATTGAG	TTAACGTTAT	GGTTTTAAAA	AAAAA			1295

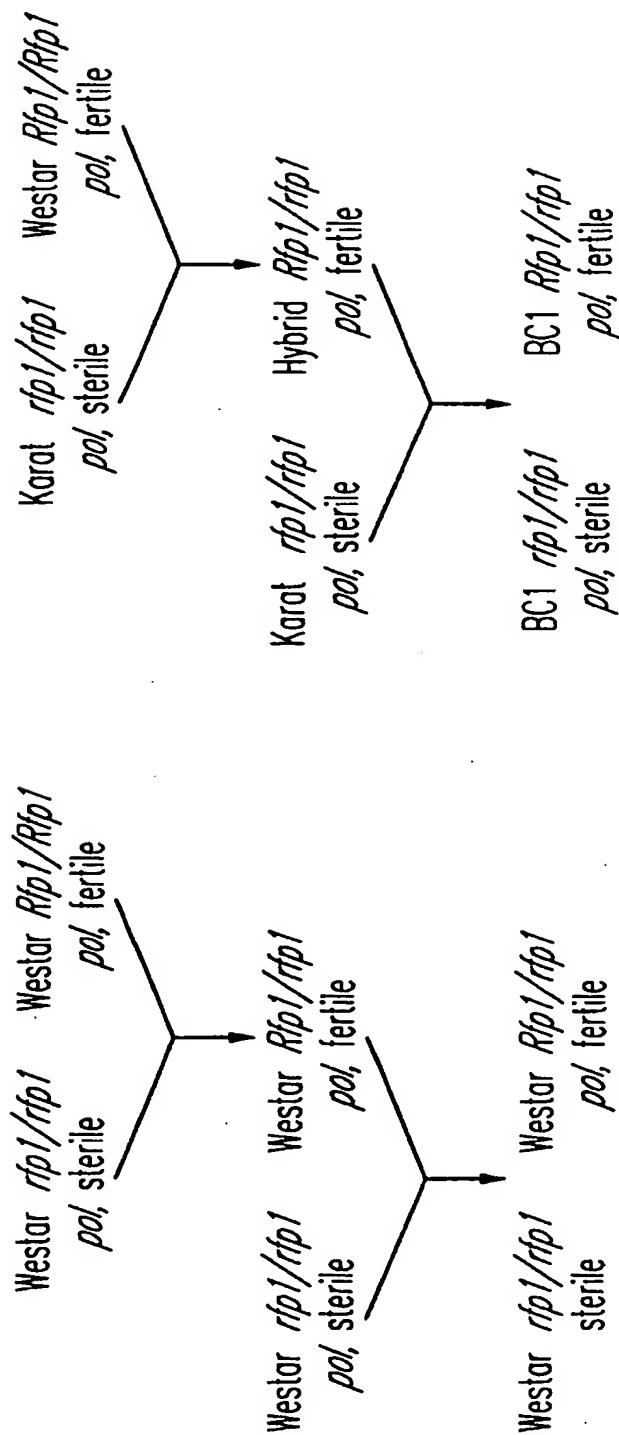
WHAT IS CLAIMED IS:

1. A probe specific for nuclear restoration of cytoplasmic male sterility of plants, which comprises a glyceraldehyde-3-phosphate dehydrogenase cDNA or genomic DNA sequence, a hybridizing fragment thereof or any DNA sequence derived therefrom for use as primers for amplification of glyceraldehyde-3-phosphate dehydrogenase, wherein said DNA sequence or hybridizing fragment thereof hybridizes to specific DNA fragments characteristic of plants possessing a nuclear restorer gene under stringent conditions.
2. A gene for nuclear restoration of cytoplasmic male sterility in plants which comprises a DNA sequence encoding glyceraldehyde-3-phosphate dehydrogenase and surrounding sequences.
3. The gene of claim 2, wherein the surrounding sequences are located 3' and/or 5' relative to the glyceraldehyde-3-phosphate dehydrogenase sequence.
4. The gene of claim 3, wherein the surrounding sequences are of about 50kb.
5. A method of production of restorer lines, which comprises genetically transforming plants with the nuclear restoration of cytoplasmic male sterility gene of claim 2.

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Crosses used to identify a marker completely linked to the *Rfp1* restorer of fertility gene. The CMS lines Westar (*pol*) and Karat (*pol*) were crossed with the restorer line Westar-Rf to generate F1 Hybrid plants, which were then crossed with the two CMS lines to generate two backcross populations. Approximately equal numbers of fertile and sterile plants were recovered (see Table 1), as expected.

FIG. 2

Comparison of *Brassica napus* cDNA clone 2NC10 with cytoplasmic glyceraldehyde-3-phosphate dehydrogenase (GAPC) cDNAs from *Sinapis alba* and *Arabidopsis thaliana*.

Brassica napus clone 2NC10 tctcgatctc atcgacaccc tct----- gatatac
Sinapis alba GAPC ----- -tttc
Arabidopsis thaliana GAPC -----ctc atcttcaacc tctctctaac tctcgttttc

41 ga-----aa tggctgacaa gaagattaag atcggaatca acggtttcgg aagaatcgg
 ga-----aa tggctgacaa gaagattaag atcggaatca acggtttcgg aagaatcgg
 gattctacaa tggctgacaa gaagattagg atcggaatca acggattcgg aagaattcgg

101 cgcttggtgg ctagagttat ccttcagagg aacgatgttg agctcgtcgc tgtaacgac
 cgcttggtgg ctagagttat ccttcagagg aacgatgttg agctcgtcgc tgtaacgat
 cgcttggttg ctagagttgt tctccagagg gacgatgttg agctcgtcgc tgtaacgac

161 cccttcatca ccaccagta catgacgtac atgtttaagt atgacagtgt tcacgggtcag
 cccttcatca ccaccagta catgacgtac atgtttaagt atgacagtgt tcacgggtcag
 cccttcatca ctactgagta catgacctac atgttcaagt acgacagtgt tcacgggtcaa

FIG. 3A

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221 tggaagcaca acgagctcaa ggttaaggat gagaagacac ttctcttcgg tgagaagcct
 tggaagcaca atgagctcaa ggtgaaggat gaaaaaacac ttctcttcgg agagaagcct
 tggaaacaca atgaactcaa gatcaaggat gagaagaccc ttctcttcgg tgagaagcca

281 gtcactgttt tcggcatcag gaaccttgag gatatgccca tggggtgagg ctggagctga
 gtcactgttt tcggcatcag gaaccttgag gatat-ccca tggggtgagg ccggagctga
 gtcactgttt tcggcatcag gaaccttgag gatat-ccca tggggtgagg ctggagctga

341 ctttgggggtt gagtctactg gtgtcttcac cgacaaggac aaggctgctg ctacttgaa
 ctttgtttgtt gagtctactg gtgtcttcac tgacaaggac aaggctgctg ctacttgaa
 ctacgttgtt gagtctactg gtgtcttcac tgacaaggac aaggctgcaa ctacttgaa

401 gggtggtgcg aagaaagtgt tcatctctgc accaagcaaa gatgctccca tgttcgttgt
 gggtggtgcc aagaaagtgt tcatctctgc accaagcaaa gatgctccta tgttcgttgt
 gggtggtgcc aagaaagtgt tcatctctga accaagcaaa gacgctccaa tgtttgttgt

461 tgggtgtcaat gagcatgagt acaagtctga tctcaacatt gttccaacg ctagt-gcac
 tgggtgtcaat gagcatgagt acaagtctga tctcaacatt gttccaacg ctagttgcac
 tgggtgtcaac gagcacgaat acaagtccga ccttgacatt gtctccaacg ctagctgcac

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621 cactaactgc cttgctccac ttgccaaaggc tatcancgac aggttttgaa ttgtcgaggg
cactaactgc cttgctccac ttgccaaaggc tatcaacgac aggttttgaa ttgtcgaggg
cactaactgc cttgctcccc ttgccaaaggc tatcaatgac agatttgaa ttgttgaggg

681 actcatgacc accgtccact ctatcactgc aactcagaag acagttgatg gtccatcaat
actcatgact actgtccact ctatcactgc tactcagaag acagttgatg gtccatcaat
tcttatgact acagtccact caatcactgc tactcagaag actgttgatg ggccttcaat

741 gaaggactgg agaggtggaa gagccgcttc ctcaacatc attcccagca gcaccggagc
gaaggactgg agaggtggaa gagccgcttc ctcaacatc attcccagca gcaccggagc
gaaggactgg agaggtggaa gagctgcttc attcaacatt attcccagca gcactggagc

801 tgccaaaggct gtcggaaaagg ttcttcacca gctc--aacg gaaagctgac cggatatgtcc
tgccaaaggct gtcggaaaagg tgcttcacca gctc--aatg gaaaattgac cggaaatgtcc
tgccaaaggct gtcggaaaagg tgcttcacca-- gctcttaacg gaaagttgac tggaaatgtct

861 ttccgtgttc ccaccgttga tgtttcagtt gttaga-ctca cggtttagact cgagaaagct
ttccgtgttc ccaccgttga tgtttcagtt gttagaccta cggtttagact cgagaaagct
ttccgtgttc caaccgttga tgtttcagtt gttagaccta ctgtcagact cgagaaagct

FIG. 3

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921 gcaacctacg atgaaatcaa gaaggctatc aaggaggaat ctgaggggcaa gctaaagggg
gcaacctacg atgaaatcaa gaaggctatc aaggaggaat ctgaggggcaa gctaaagggg
gctacctacg aagaaatcaa aaaggctatc aaggaggaat ccgaaggcaa actcaagggg

981 atccttgggtt acacagagga tgatgttgtc tcaaccgact tcgttgggtga caacaggtcg
atccttgggtt acacagagga tgatgttgtc tcaactgact tcgttgggtga caacaggtcg
atccttgggtt acacagagga tgatgttgtc tcaactgact tcgttgggtga caacaggtcg

1041 agcatttttg acgcaaaggc tggaaatcgcg ttgagtgaca actttgtgaa gctggtgtcg
agcatttttg acgcaaaggc tggaaatcgca ttgagtgaca acttcgtgaa gctggtgtcg
agcatttttg acgcaaaggc tggaaatgca ttgagcgaca agtttgtgaa attggtgtca

1101 tggtagcaca acgaatgggg ttacagtacc cgtgtggtcg acttgatcat tcacatgtcc
tggtagcaca acgaatgggg ttacagtacc cgtgtggtcg acttgatcat tcacatgtcc
tggtagcaca acgaatgggg ttacagtacc cgtgtggtcg acttgatcat ccacatgtca

1161 aaggccctaa tcgatga--- --agatctcg agtgat--gt aatgg-----
aaggccctaaa acgctga--- --agatctac aatgat--gt aatgg-----
aaggccctaaa --gctaagaa gcagatctcg aatgataggg agtggaaagt catctgttca

FIG. 3

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1221 ----- tgttttttaa ttggtgtttt tatcgaataa attttct-tg ggttttgaaa
----- tg-tcttaaat ttgtgggtttt ---cgaataa gatttctttg gg-----
tccccttta tgggtctgaat ttgtcgtttt ---cgaataa aatttctttg aacttggaa-
1281 cctttatgg- -ttttgg--- --cgaattct ctactttcac gtgacgtgat aagaagtgtg

-ctttttttt tttttgggtt tcttaattct ca---ttcat gtgagggtgat -gggaggttg
1341 tagaccgggt gttttttatt ttactga-- -----

tagaccg-at g-----t ttactggaa gccctttgtt ttgggtttt gatataattga
1401 -----

gttaacgtta tggtttttaa aaaaaa

Final

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Polymorphism detected by cRF1 probe in *Brassica napus* and a genetic population segregating for the *Rfp1* gene. Fertile plants have the *Rfp1* gene, sterile plants do not. Arrow indicates the polymorphic restriction fragment associated with fertility restoration.

RI=Restorer line parent
F=fertile plant
S=sterile plant

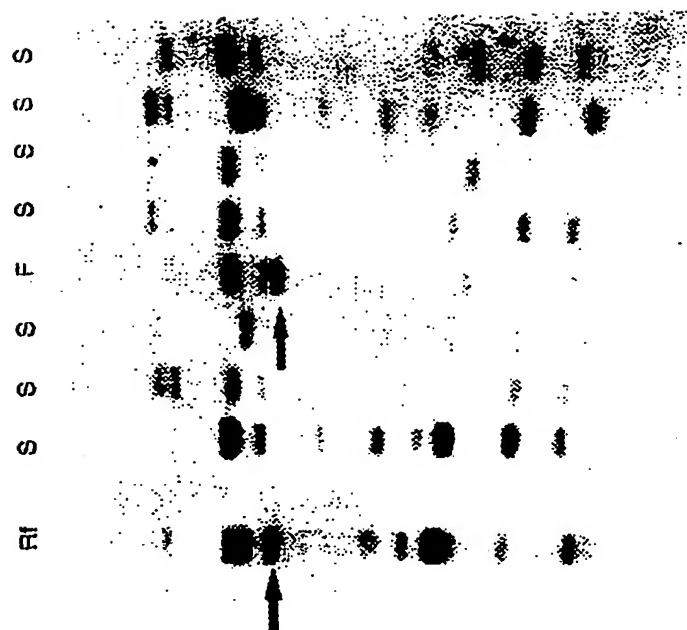


FIG. 4

INTERNATIONAL SEARCH REPORT

Inta. .ional Application No

PCT/CA 97/00424

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/68 C12N15/82 C12N15/53 C12N15/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SINGH M ET AL: "Nuclear genes associated with a single Brassica CMS restorer locus influence transcripts of three different mitochondrial gene regions" GENETICS, vol. 143, no. 1, May 1996, page 505-16 XP002043618 see the whole document ---	1
A	WISE R ET AL: "mapping complementary genes in maize: Positioning the RF1 and RF2 nuclear-fertility restorer loci of texas (T) cytoplasm relative to RFLP and visible markers" THEORETICAL AND APPLIED GENETICS, vol. 88, no. 6-7, 1994, pages 785-95, XP002043619 see the whole document ---	1

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☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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Date of the actual completion of the international search

15 October 1997

Date of mailing of the international search report

29.10.97

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Fax: (+31-70) 340-3016

Authorized officer

Osborne, H

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 97/00424

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claims No.
A	DELOURME R ET AL: "Identification of RAPD markers linked to a fertility restorer gene for the Ogura radish cytoplasmic male fertility of rapeseed (Brassica napus L.)" THEORETICAL AND APPLIED GENETICS, vol. 88, no. 6-7, 1994, pages 741-48, XP002043620 see the whole document	1
A	SCHNABLE P ET AL: "Recovery of heritable, transposon-induced, mutant alleles of the RF2 nuclear restorer of T-cytoplasm maize" GENETICS, vol. 136, no. 3, 1994, pages 1171-85, XP002043621 see the whole document	1